# SHORTER ARTICLES AND DISCUSSION

## STERILITY[1]

THE problem of sterility presents many points of interest to the biologist, whether he be a clinician, breeder of plants or animals or a pure biologist, both from a descriptive and an experimental aspect. By the physician, the failure of individuals to produce children in marriage is designated by this name, although the causes may be widely at variance in different cases. Thus, the failure may be due to the impotence of the male, induced by a variety of causes, such as congenital impotence, where sex cells are not formed, although the organs themselves may be apparently normal; or evident deformities may occur such as the failure of one or both testes to descend (cryptorchism); again, impotence may be induced by disease, such as gonorrhœa, syphilis and the like; or again, through presenile debility caused by excessive activity of the organs concerned. On the other hand, the failure to produce offspring may be due to the corresponding impotence of the female, or it may result from wholly secondary causes, such as the failure of the ovum to become fixed. All of these cases are grouped, collectively, under the term sterility. Very frequently a simple operation upon the uterus of the female is sufficient to cause the ovum to become fixed, so that subsequent ovulations, accompanied by fertilization, result in offspring and the sterility disappears. With congenital sterility, where no sex cells are formed, the case is obviously different, for the condition there is permanent. Cases of congenital sterility seem to be rare, although it is exceedingly difficult to obtain concise data upon the subject. The impotence caused by disease may, in time, disappear, especially if the ravages of the disease have not destroyed the germ cells, as is often the case. An impotence ascribed to psychical causes may rarely occur, but concerning this factor, we have, obviously, little or no exact evidence.

The sterility induced in crossing animals and plants belonging to different varieties and species has long been known. The

[1] Presented at the fourth evening seminar of the Harpswell Laboratory, August 2, 1910.

early hybridists of plants, Gärtner, Kölreuter, Nägeli, Gordon and others, were familiar with the frequent failure of pollen, placed upon the stigma of certain plants, to produce seed in the ovules of that plant, and with the fact that even if seed is obtained, it may not grow when planted. Kölreuter and Gärtner saw in sterility the criterion whereby species may be distinguished. If two forms bred together produced seed, and the seed was capable of growing into perfect plants, the two forms were considered as belonging to the same species. If, on the other hand, no seed was produced, or if produced, this seed was incapable of growth, the two forms were considered as belonging to distinct species. However, as Darwin points out, these two workers were biased in their appreciation of sterility, as a valid criterion for distinguishing species, for they themselves, as have indeed all workers since them, described all degrees of sterility under such circumstances, from slight deviations in form of the hybrid plants, through a condition where the seed, although formed, was shrivelled and incapable of producing the young plant, to complete sterility, where no indication of even a pollen-tube is seen in the style of the pistil. Obviously, the personal equation of the experimenter will be a potent factor in determining whether there were two species, or a single one involved in questionable cases, and the definiteness of the criterion is largely detracted. To Darwin, who had carried out thousands of crosses, the use of sterility as a distinguishing character for species was an impossibility, and this fact was utilized by him in combating the criticism urged against the theory of natural selection, that it could not account for the origin of sterility by assuming that it was a factor in isolating species.

It has been only within comparatively recent times that an understanding has been reached as to what are the intimate results of sterility. It is true that Gärtner knew that the pollen of many hybrids was shrivelled and functionless, but farther than this, nothing was known of the condition of the spore-bearing and gamete-bearing parts; for the rôle of the pollen-tube, with the nuclei contained, and of the ovum in the ovary was as yet unknown and the discovery of the alternation of generations in the higher plants had not been made. Not until the reduction of the tetrads had been worked out was it possible to understand the failure of the hybrid to produce seed, or, if seed were produced, to understand why abnormalities occurred in the young hybrid plants.

We have spoken thus far mainly of the work of botanists, for the reason that little or nothing has been accomplished by zoologists until recently, which throws light upon the problem of sterility. It was known by Aristotle that species would cross and that the offspring varied from the parents in vigor and in other characters. If the etymologists inform us correctly, the word leopard, compounded of *Leo,* lion, and *Pardos,* panther, or tiger, points to a belief among the Greeks, that the leopard was a cross between the lion and tiger. The knowledge that captive animals bred only rarely, many not at all, and that even when mating occurred, the act was functionless and no offspring resulted, although the animals were apparently normal, is nothing of recent date. As to why offspring are not produced under these conditions, we are wholly ignorant. No one has determined whether the male actually conveys the fertilizing fluid to the female and if so, whether functional spermatozoa are present. It is known that in some cases, confinement of wild animals induces degeneration of the internal generative organs. Psychic causes, too, are undoubtedly present, inhibiting the mating instinct, but these factors are so subtile that they defy analysis. The data are familiar to every keeper of zoological gardens, and yet no attempt, to my knowledge, has been made to determine any of the details involved.

Several cytologists have examined the gonads of hybrid animals with a view to determining the condition of the sex cells. Thus, Guyer studied the crossed pigeons from the cotes of Professor C. O. Whitman at Chicago and found great abnormalities in the secondary spermatocytes, such as clumping of the chromatin, degeneracy of the cells as a whole, tri- and other abnormal mitoses. Spermatozoa were formed in many cases, but these cells were obviously abnormal and pathologic. The gonads of the mules obtained by crossing canaries and English goldfinches, siskins, bullfinches, etc., are degenerate, and in some cases which I have examined no trace of gonad could be found.

The cytology of the gonad of the mule obtained by crossing the mare with the jackass has been examined by H. E. Jordan who describes the testis as follows:

> The seminiferous tubules are lined with Sertoli cells, spermatogonia and a few primary spermatocytes in early stages. The nucleus of the latter appears to be in the spireme phase of the contraction stage and in process of regressive change. Mitoses are exceedingly rare and those

present are seen among the basal cells. Nothing corresponding to a secondary spermatocyte or a spermatid can be found, nor are spermatozoa anywhere present, either in the seminiferous tubules or the epididymis. The absence of spermatozoa explains why mules are infertile *inter se,* as also the fact that no issue results from a cross between a female horse and a male mule (the cross between a female mule and a stallion is known to have resulted in offspring).[2]

Aside from this study, and that of Guyer spoken of above, I know of no examination which has been made of the gonads of hybrid vertebrated animals. The conditions which I have found in canaries resemble closely those found by Guyer, in pigeons. The observations of Ancel and Bouin[3] on cryptorchid horses resemble to quite an extent, those by Jordan upon the mule testis.[4]

Hybridization experiments with lower forms of animals throw some light upon the behavior of the germ cells concerned in fertilization. Moenkhaus found that he could cross *Fundulus* and *Menidia,* two species of fishes, and obtain normal hybrids. Owing to the differences in size of the chromosomes in the two species, he was enabled to determine the fate of the chromosomes of the egg and of those of the spermatozoon and it was possible to follow them through the embryonic history, through the various mitoses of segmentation and later. Here, the chromosomes were in no way antagonistic and proceeded through the mitoses side by side.

Balzer[5] performed a series of very interesting experiments upon several species of echinoderms occurring in the Bay of Naples. He used four species, *Strongylocentrotus, Echinus, Arbacia* and *Sphærechinus,* making reciprocal crosses. Crosses between *Strongylocentrotus, Echinus* and *Arbacia* gave normal fertilizations, with no loss of chromatin in the earlier segmentation anaphases, and the plutei exhibited normal skeletons, except

[2] Whitehead, R. H., 1908, "A Peculiar Case of Cryptorchism and its Bearing upon the Problem of the Function of the Interstitial Cells of the Testis," *Anatomical Record* (Philadelphia, U. S. A.), Vol. 2, p. 177.

[3] Ancel et Bouin, 1903–4, *Journ. de Physiol, et de Path.,* T. 6, Nr. 6, 7. Also *Comptes Rendus,* T. 137, Nr. 26; T. 138, Nrs. 2, 3, 4.

[4] See Schwalbe, E. (Herausgeber), "Die Morphologie der Missbildungen des Menschen und der Tiere," Ein Hand- und Lehrbuch, Dritter Theil: Die Einzelmissbildungen.

[5] Balzer, F., 1909, "Ueber die Entwicklung der Echiniden-Bastarde mit besonderer Berücksichtigung des Chromatinverhältnisse," *Zool. Anz.,* Bd. 35, S. 5.

in one case. With *Sphærechinus* and *Strongylocentrotus* several chromosomes were eliminated in the anaphase of the first segmentation stage, which were excluded from the nucleus of the resulting cells, when the nuclear walls were formed. Correlative with this, the plutei showed abnormalities and the general resemblance was to the maternal species. Apparently here the case is different from that of Moenkhaus's *Fundulus-Menidia* hybrids, for the chromatin of the egg in part at least, is not adapted to association with that of the eggs of the other species. If this interpretation is correct, we may refer the abnormalities of the hybrid produced from the cross of *Sphærechinus* to the loss, during the segmentation stages, of chromosomes derived from the female. The analysis cannot be pushed farther back, in this case, for we are unable to understand why these chromosomes which are excluded from the reconstructed nucleus are "incompatible" with those of the egg-nucleus. Whatever the cause, it is probably similar to the agglutinization of erythrocytes, spermatozoa, bacteria and other cells in the fluids from other organisms or in artificial media. In this connection, it is interesting to recall that Guyer and Jordan found the abnormalities in the testes of hybrids appearing first during the synapsis period, when the chromosomes from paternal and maternal sources conjugate two-by-two, either end-to-end or side-by-side (probably side-by-side in chordates, according to the observations of Winiwarter). Recent study of spermatogenesis and oogenesis points to the conclusion that the maternal and paternal chromosomes remain distinct and more or less isolated from one another in the primary germ cells, from the time of fertilization until synapsis, and then for the first time are they intimately associated into pairs, as Montgomery suggested —a view which has been abundantly confirmed by Sutton, Stevens, Wilson, and a number of others. Here, then, would it be expected that incompatibilities, if ever present, would become apparent. If the phenomenon is of the same category as agglutinization, hemolysis and the like, it should be possible to render the sex-cells of one animal immune to the lytic action of those of other animals, on the principles of immunization and antibody development. In a case, however, where such a relation appears much more clearly, it seems that the phenomena are not of the same kind. I refer to the auto-immunity of *Cynthia*, where the eggs of a given individual of *Cynthia partita* cannot

be fertilized by the spermatozoa of the same individual. Morgan analyzed the phenomenon in the light of immunity and was unable to demonstrate a parallel between the *Cynthia* immunity and that in anti-body formation.[6] However, the case described by Morgan may not be equivalent to those of hybridization experiments, such as we have described.

Concerning the relation of the chromosomes to fertilization and subsequent condition of the embryo, Bataillon[7] derives certain evidence from his amphibian crosses for the conclusion that the number of chromosomes in the two parent species involved in the cross is of importance in determining the condition of the embryo resulting. Thus, when the number of chromosomes in the two species is the same, no embryo results, while crosses between species with different numbers of chromosomes lead to progeny. If this observation is true, it might be due to the fact that mainly, or only those chromosomes which are in excess of the number occurring in the species with smaller number of chromosomes and which do not pair up in synapsis with the chromosomes of that species, are functional in producing the embryo. Under these conditions, we should expect that the embryo would resemble the species with the greater number of chromosomes. However, this does not follow, inasmuch as Bataillon did not observe nuclear copulation, but rather a mode of artificial parthenogenesis is supposed to occur, the male element being wholly without effect in inducing fertilization and, therefore, the progeny would resemble the maternal species.

In 1906, Emil Godlewski, Jr., succeeded in obtaining a few hybrids between the sessile crinoid, *Antedon* ♂ and *Echinus* ♀, thus involving two classes of echinoderms, the Echinoidea and Crinoidea. The sperm exerted a marked inhibition of development as a whole and the hybrids resembled the maternal *Echinus*. This case is probably similar to the one described above where non-compatibility is evident between egg and sperm nuclei in fertilization. Godlewski did not examine the chromosomes with a view to determining their condition in the cells of the hybrids.

[6] Morgan, T. H., 1910, "Eggs of *Cynthia*, Immune to their own Sperm," a paper before the Society for Experimental Biology and Medicine, to be published in full in the *Journal of Experimental Zoology*.

[7] Bataillon, M. E., "Le substratum chromatique héréditaire et les combinaisons nucleaires dans les croisements chez les Amphibiens," *Comptes Rendus*, Paris, T. 147, f. 692.

Godlewski did not find it necessary to resort to artificial means in causing the spermatozoon of *Antedon* to penetrate the egg of *Echinus*, but by an ingenious method Loeb[8] was enabled to cause the egg of a member of one phylum to be fertilized by the spermatozoa from a member of a different phylum. Kupelweiser[9] afterwards applied Loeb's method to a cross between the mussel, *Mytilus* ♂, and the echinoderm, *Echinus* ♀, the method involving a subjection of the eggs to a hypertonic solution of sea-water, as in artificial parthenogenesis. Bataillon[10] believes that only artificial parthenogenesis is operative in this case, basing his conclusions partly upon analogous crosses in amphibians, where he has crossed *Triton* ♂ with the toad *Pelodytes* ♀, and observed that no sperm asters formed and segmentation proceeded without any participation on the part of the sperm nucleus.[11] Of course if Bataillon's contention is correct, the case is of no interest in the present connection, but if the two cases are not parallel, Loeb has overcome what we may grossly term sterility, by artificial means, by modifying the osmotic pressure and the permeability of the egg-membrane so that the spermatozoon of the foreign species may enter. Sterility here then may be due to purely mechanical factors. Yatsu has pointed out that the failure to procure crosses between the frogs *Rana sylvatica* ♀ and *Rana virescens* ♂ is due to the fact that the heads of the spermatozoa of the latter are too large to enter the eggs of the former. Here, again, a purely mechanical factor is involved.

While no experiments have been performed to my knowledge to determine whether a kind of acclimatization may be induced in hybrids, yet some experiments performed by botanists shed some light upon this point. Gärtner,[12] Wichura[13] and Nägeli[14] believed that fertility decreased in later generations, but Naudin,[15] C. C. Hurst[16] and others, including DeVries,[17] believe

[8] Loeb, J., *Roux's Archiv*, Bd. 26, Heft 3.

[9] Kupelwieser, H., *Roux's Archiv*, Bd. 27, S. 434.

[10] Bataillon, E., ''L'imprégnation hétérogène sans Amphimixie nucléaire chez les Amphibiens et les Echinodermes (à propos du recent travail de H. Kupelwieser),'' *Roux's Archiv*, Bd. 28, S. 43–48, 1909.

[11] Bataillon, E., ''Imprégnation et Fécondation,'' *Comptes Rendus*, 11 Juin, 1906.

[12] Gärtner, 1849, ''Bastarderzeugung im Pflanzenreich.''

[13] Wichura, M., 1865, ''Die Bastardbefruchtung im Pflanzenreich.''

[14] Nägeli, C. v., 1866, ''Botanische Mittheilungen,'' *Sitz. ber. der Münch. Akad. Wiss.*, 13 Jan., 1866.

[15] Naudin, Ch., 1869, ''Nouvelles recherches,'' *Nouvelles Archives du*

that fertility increases, or, in other words the barrier, more or less perfect, between hybridization of the species involved, is gradually broken down. As Kerner[18] has shown, indeed, many hybrid plants are more fertile than the parent species and this is maintained and increased during future generations, so that the hybrids have replaced the parent species. There is, then, a basis for believing that a kind of acquired "congeniality" obtains, whereby the conjugating cells become more compatible, whatever this may ultimately involve. An interesting case was described by Gordon[19] where *Nicotiniana, Digitalis* and other hybrids are sterile *inter se,* but fertile with the parent forms. In the cross between the dog and the wolf, sterility begins, not in the earlier generations, but in later ones, according to the observations of Flourens, but as Darwin remarks, it is doubtful that this is due to increasing sterility because of crossing, but rather to confinement or, indeed, to inbreeding.

The students of plant cytology were the first to examine critically the structures concerned in reproduction in hybrids. Gärtner long ago observed the shrivelled pollen grains, as we have said before, but that was before the modern cytological methods came into use. Jančič[20] has contributed widely to this department of research. He observed that the number of pollen grains decreased in certain hybrid plants and that in the several species he examined, this numerical reduction occurred in approximate amounts to one fourth, one half or three fourths the average number of grains. Moreover, cytological examination of the anthers showed that many of the nuclei after reduction of the tetrads were atrophied, and the conclusion is obvious that herein lies the explanation of his numerical ratios mentioned above; for in some crosses, three fourths of the nuclei destined for the pollen (the daughter and granddaughter cells of the pollen mother-cell), or, in other words, three of the four tetrads, aborted, leaving but one to become pollen (hence the one fourth), while in other cases, two of the tetrads disappeared (two

*Muséum a'histoire naturelle,* Paris.

[16] Hurst, C. C., 1900, *Journ. Roy. Hortic. Soc.,* Vol. 24, p. 124.

[17] De Vries, H., 1903, "Die Mutationstheorie," Vol. 2, p. 66.

[18] Kerner, A. von, "Können aus Bastarden Arten werden?" *Oesterr. Bot. Zeit.,* Bd. 21, 1871.

[19] Gordon, D. A., 1862, *Mem. Acad. Stanisl.,* p. 228.

[20] Jančič, A., "Untersuchungen des Pollens hybrider Pflanzen," *Oesterr. bot. Zeitschr.,* 1900, Nr. 1, 2, 3.

fourths), so that one-half of the normal number of pollen grains appeared. Here, again, it is the period of synapsis or of conjugation of the maternal and paternal chromosomes (the stage corresponding to the gametophyte, where the chromosomes are reduced in number) where the effects of hybridization are evident.

Juel[21] has shown that the chromatin distribution in the nuclei of hybrids is irregular, corresponding to the conditions in hybrid animals described earlier in the present paper. The condition of the chromosomes during actual synapsis is such that it is impossible to observe their behavior directly and, doubtless, nothing could be learned of the ultimate cause of the irregularities through mere observation during these periods, even if the chromosomes could be more clearly followed. In such cases, it seems to me, we have fought the matter of sterility to its inner keep, and until experimentation comes to our aid, we shall be able to proceed no farther. "Über die Art und Weise, wie die Eizellen steril werden, haben wir keine erwähnenswerthen Angaben gefunden" (DeVries).

Several cytologists have taken advantage of the exhaustive studies of DeVries upon hybrids of the primrose, *Œnothera lamarckiana,* to study the cellular phenomena of crosses whose general features are well known. Thus, Geerts[22] has recently studied the partial sterility and the development of the embryosac of *Œnothera* and finds that there are no antipodal cells developed and that the endosperm forms from the pole cells. Sterility, says Geerts, is a matter of irregularities in the reduction division and therefore his observations tally with those, both plant and animal, spoken of above.

Gates[23] and Miss Lutz[24] have investigated the cytology of *Œnothera* crosses and of *Drosera* hybrids. Irregular distribution of the chromosomes obtained in the reduction division and these differences are correlated with the external characters of the crosses.

[21] Juel, H. O., "Beiträge zur Kenntnis der Tetradentheilung," *Jahrb. wiss. Bot.,* Bd. 35, 1900.

[22] Geerts, J. M., "Récueil Trav. Bot. Néerl.," T. 5, 1909.

[23] Gates, R. R., *Bot. Gaz.,* Vols. 46 and 48; *Science,* Vols. 27 and 30; *Archiv für Zellforsch.,* Bd. 3, H. 4, 1908–09.

[24] Lutz, Annie M., *Science,* Vol. 29, 1909.

Finally, Rosenberg[25] has invaded this fascinating field and studied the hybrid sundews exhaustively. The main results of his observations are similar to those given above in the case of the other workers. Rosenberg adopts the explanation of "Unverträglichkeit" (incompatibility) for the behavior of the chromosomes—a view which Tischler,[26] who speaks from much experience in the cytology of hybrids, believes to be inadequate, for the reason that, as Rosenberg as well as he himself shows, there are a few cases where typical embryo-sacs are developed and indeed the *anlage* of the young sporophyte may be found. "Die gewöhnliche Sterilität nicht in einer Unverträglichkeit der beiderelterlichen Chromosomen liegt."[27]

We are able to see, therefore, that sterility, as far as may be judged from studies upon the germ cells which have been made thus far, is a matter of the fundamental constitution of the organism. It concerns the bearers of hereditary traits, the chromosomes. All of the studies which have been made point to the conclusion that whatever may be its nature, there is an "incompatibility" existing between the chromosomes of individuals of different species or varieties. Tischler's contention seems to me to be ill-founded, for the cases where normal structures occur in the embryo-sacs of these hybrid plants are exceedingly few, and when we consider the observations of other workers upon plant material, and of the animal cytologists we find abundant reason to believe that the exceptions mentioned by Tischler may be better explained in another way. Moreover, we know nothing of the constitution of the nuclei of the cells of the young sporophytes, with respect to the mingling of maternal and paternal chromosomes. It may be, indeed, as Gates has suggested, that a condition of apogamy may obtain.

Undoubtedly the next few years will see many points as yet undetermined, brought into proper perspective and we shall be able to give a more complete account of the rationale of sterility.

MAX MORSE.

[25] Rosenberg, O., "Cytologische und morphologische Studien an *Drosera longifolia* by *rotundifolia*," *K. Vetenskaps Akad. Handl.*, Bd. 43, Nr. II., 63 S. Stockholm.

[26] Tischler, G., *Zeitschr. f. ind. Abstammungsl.*, Bd. 3, H. 3, 1910.

[27] Tischler, *l. c.*